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THE EFFECT OF pH AND GROWTH PHASE ON HEAT SHOCK INDUCED THERMOTOLERANCE IN *LISTERIA MONOCYTOGENES* AS MEASURED IN A BROTH AND A MINCED BEEF SYSTEM

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SUMMARY

The effect of heat shock on thermotolerance (D_{60}-values) of log phase and late stationary phase cultures of *Listeria monocytogenes* was compared at different pH's (5.4, 5.8 and 6.2) in tryptic phosphate broth and in minced beef. In order to simulate conditions in minced beef, lactic acid (90 mmol l^{-1}) was added to the broth. The influence of heat shock on thermotolerance was similar in beef and broth for log phase cells but less consistent in late stationary phase cells. At low pH (5.4), thermotolerance in log phase cells did not show a statistically significant increase upon heat shock in beef or broth. With increasing pH, however, a gradual increase in heat shock induced thermotolerance was observed in both beef and broth. In stationary phase cells heat shock provided very little or no significant increase in thermotolerance.

INTRODUCTION

Listeria monocytogenes is an organism of prime concern with regard to the production of cook-chill foods due to its psychrotrophic nature, severity of disease, widespread occurrence and relatively high heat resistance compared to other foodborne pathogens (Sofos 1993; Schofield 1992). Thermotolerance of *L. monocytogenes* can be even higher if exposed to sublethal temperatures slightly above the optimum growth temperature (Quintavalla and Barbuti 1989). In the production of sous-vide cooked beef roasts exceptionally low heating temperatures and long come-up times are employed (Hansen *et al.* 1995) and such a process has been suggested to resemble a heat shock temperature profile (Farber and Brown 1990). In this respect, *L. monocytogenes* is of concern because it is frequently found in fresh beef products and if exposed to such heat shock inducing temperatures may develop sufficient thermotolerance to survive the process.

rber and Brown (1990) observed no significant increase in thermotolerance of *L. nocytogenes* after a heat shock (48°C, 30 min) in a pork/beef mixture whereas a 2.5-fold ease was noted by Linton *et al.* (1990) after a heat shock (48°C, 10 min) in broth. Such ies indicate a considerable difference in the relative increase in thermotolerance of *L. ocytogenes* when heat shocked in meat compared to when heat shocked in broth.

factors seem to affect the extent to which the cells become more thermotolerant after shock. Recently, pH and the presence of weak acids has been shown to be important heat shock induced thermotolerance in yeast (Cheng and Piper 1994).

growth phase has also been implicated as a significant factor for the relative e in thermotolerance upon heat shock (Watson 1990).

pose of the present work was to determine the effect of interactions between pH and

growth phase on heat shock induced thermotolerance at 60°C in *L. monocytogenes* and to compare this effect in a broth and a food model system.

MATERIALS AND METHODS

Organism and Culture Conditions

L. monocytogenes (serotype 1) isolated from pasteurized cured ham was obtained from The Danish Veterinary Service, Food Control Laboratory. The strain was maintained as 200µl frozen stock cultures at -80°C. Cultures were grown at 30°C with shaking at pH 7.3 in 100 ml tryptic phosphate broth (TPB; Conner *et al.* 1986) to log phase (14 h) or late stationary phase (36 h).

Broth Model System

pH-treatment and Heat Shock Procedure. Portions of 20 ml, from the respective cultures, were spun down and resuspended in 1 ml of fresh TPB for 15 min at 20°C. Nineteen ml volumes of TPB at pH 5.4, 5.8, 6.2 and 7.0 containing 90 mmol l^{-1} lactic acid (90%, Struers) were then added; a typical lactic acid concentration for beef (Newbold and Scopes 1967). These cultures were kept for 20 min at 20°C in order to simulate the procedure used in beef experiments (see below). Subsequently, heat shocked cells received a treatment of 30 min at 46°C, while non-heat shocked cells were heated immediately at 60°C.

Thermal Inactivation. Broth suspensions were heated at 60°C in a submerged-coil heating apparatus (Cole *et al.* 1993). Samples of 0.2 ml were taken at predetermined time intervals except where low cell numbers were expected when 0.6 ml were removed. These samples were dispensed directly into TPB (5 ml, 20°C) ensuring near to instantaneous cooling of the samples.

Minced Beef Model System

pH-adjustment. Fresh beef (*M. semitendinosus*) was deep fried for 0.5-1 min and the cooked exterior aseptically removed. The meat was then cut into smaller blocks before being passed through a 0.4 cm holeplate in a sterilized food processor. Batches of minced beef were maintained under frozen storage (-18°C) until further use. The meat was thawed overnight at 3°C before pH adjustment was performed. pH was measured using a direct insertion probe electrode (LOT406-M3-S7/25 INGOLD) and a pH-meter (Knick 750). pH was adjusted to 5.4, 5.8 and 6.2 in slurries of meat and deionized water (1:1). The volumes of NaOH (1M) or HCl (1M) needed to obtain the pH-values were determined and appropriate solutions were made and added to minced beef. The meat was kept overnight at 3°C and pH measured the following day prior to use. Portions of minced beef (10±0.2 g) were weighed into individual vacuum pouches and shaped into a meat block.

Inoculation and Heat Shock Procedure. Cell suspension for meat inoculation was spun down, resuspended and diluted in fresh TPB to obtain a density of approx. $5x10^7$ colony forming units (cfu) per ml. About 15 min elapsed from the time of resuspension until the start of inoculation. 200 µl of cell suspension was injected into the centre of each sample which were evacuated and sealed. The preparation time was approx. 20 min. Meat samples for heat shocking were heated for 30 min at 46°C (core temperature) while non-heat shocked samples were heated immediately at 60°C. The inoculum level was determined from two uncooked inoculated samples.

Thermal Inactivation. Meat samples were placed in a rack with no contact between the samples and immersed in circulating hot water at 60°C. During heating, core temperatures in two meat samples were continuously monitored using thermocouples and a data logger (Grant 1200 series Squirrel meter/logger). The heating time required for the samples to reach an internal temperature of 60°C was 6 min. At different time intervals duplicate samples were removed, placed in a water bath at 10°C for 10 min and subsequently held on ice until analysed. The internal temperature of the samples cooled to below 30°C in approx. 2 min. The samples were diluted ten-fold in TPB and homogenised for 60 s.

Enumeration of Survivors

Survivors were counted following serial dilution in physiological saline + 0.1% Bacto-peptone (

surface plated on tryptic soy agar (Difco) + 0.6% yeast extract (Difco) + 0.1% sodium pyruvate (Sigma). In beef experiments, when low counts were anticipated, a five tube most probable number (MPN) analysis was performed in a corresponding medium without added agar. Plates and tubes were incubated at 30°C for 5 d. Material from positive MPN-tubes were streaked onto *Listeria* selective agar Oxford formulation (Oxoid) and incubated for 24 h at 30°C for verification.

Data Analysis

Estimation of heat resistance. Heat resistance was expressed as D_{60}-values calculated as the absolute value of the inverse slope of the least square regression lines fitted to $\log_{10}$ of cfu. In the beef experiments pasteurization values were calculated from the registered heating time and temperature using the equation described by Shapton and Shapton (1991) and a z-value of 6.9°C, calculated from compiled data of Mackey and Bratchell (1989). The pasteurization values were used as the independent variable in the linear fit.

Statistical analysis. Statistical analysis was performed separately on data from broth and beef experiments. For each growth phase the D_{60}-value was used as the response variable in analysis of variance with heat shock and pH as fixed effects. Least squares estimates of marginal means are presented.

RESULTS

Thermotolerance (D_{60}-values) of *L. monocytogenes* was 2 to 5-fold higher in minced beef than in broth. The highest difference was observed at pH 6.2 (Fig. 1A+B). In broth the effect of pH on thermotolerance in non-heat shocked cells was neglible but, especially in stationary phase cells, thermotolerance increased slightly in beef at high pH (Fig. 1B).

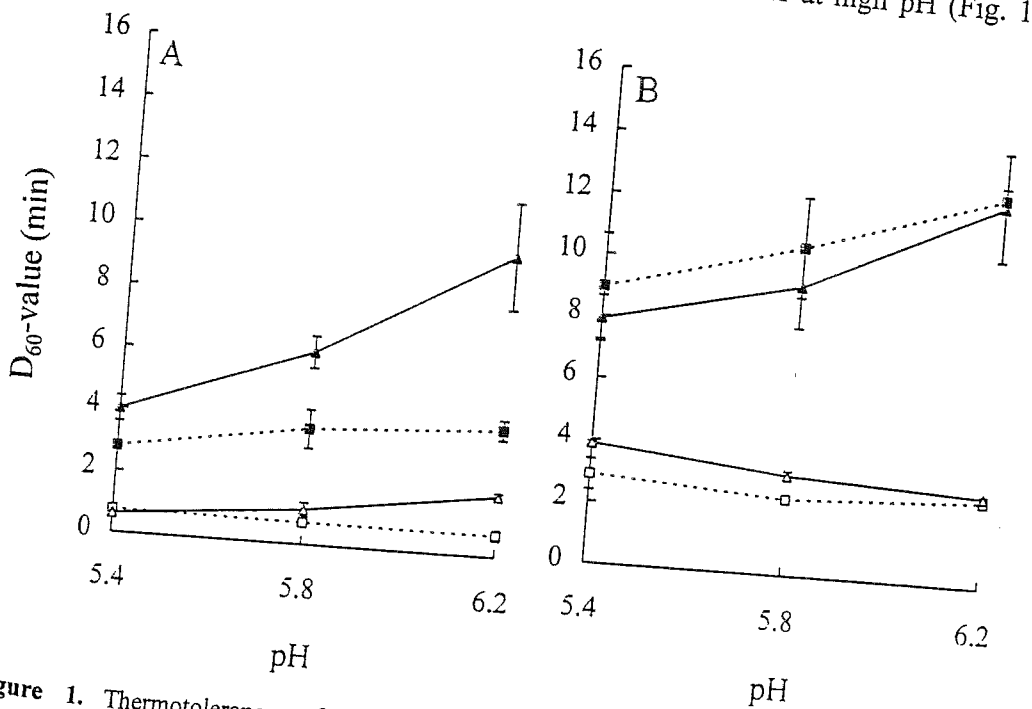

Figure 1. Thermotolerance of log phase (A) and late stationary phase (B) *Listeria monocytogenes* in minced beef (full symbols) and broth (open symbols) before (dotted lines) and after (full lines) a heat shock at 46°C in 30 min.

The relative influence of heat shock on thermotolerance was similar in minced beef and broth although less consistent for late stationary phase cells (Table 1). Heat shock induced

very little or no statistically significant increase in thermotolerance in stationary phase cells (Table 1). In log phase cells, thermotolerance did not increase after heat shock in beef or broth at pH 5.4, however, at elevated pH heat shock induced thermotolerance increased in both beef and broth (Fig. 1A). At pH 7 in broth this trend was further supported by a 5.7-fold increase in thermotolerance upon heat shock for log phase cells (Table 1).

Table 1. Increase in thermotolerance (D_{60}-value) due to heat shock of log and late stationary phase *Listeria monocytogenes* in minced beef and tryptic phosphate broth with 90 mmol l^{-1} lactic acid added.

		Growth phase	
Heating menstruum	pH	Log	Late stationary
Broth	5.4	0.8	1.3
	5.8	1.6	1.3
	6.2	2.8	1.1
	7.0	5.7	1.0
Minced beef	5.4	1.4	0.9
	5.8	1.7	0.9
	6.2	2.4	1.0

The heat shock (46°C for 30 min) applied in the present study caused a statistically significant loss in viability (Table 2) for log phase cells in beef with a pH of 5.4, but a similar effect could not be observed in broth.

Table 2. Loss in viability (log_{10}cfu) of *Listeria monocytogenes* during heat shock (46°C, 30 min) in minced beef and tryptic phosphat broth with added lactic acid (90 mmol l^{-1}).

		Growth phase	
Heating menstruum	pH	Log	Late stationary
Broth	5.4	$-0.01^{b,c}$	-0.05^{c}
Minced beef	5.4	1.13^{a}	$0.04^{b,c}$
	5.8	0.14^{b}	-0.02^{c}
	6.2	-0.04^{c}	$0.02^{b,c}$

[a-c] Different superscripts denote statistically significant differences ($P \leq 0.5$)

DISCUSSION

The effect of heat shock on thermotolerance has received much interest but the practic importance of the phenomena in food processing has not been resolved. Results from

present study show that the interaction between heat shock and pH is almost similar in beef and broth (added lactic acid) for log phase *L. monocytogenes*. The effect of this interaction on thermotolerance is, however, in some cases different for cells in late stationary phase in beef and broth. These findings may explain some of the inconsistencies found in the literature. They also suggest that bacterial growth phase becomes an important factor when attempting to predict the effect of heat shock on thermotolerance in foods on the basis of results obtained in a broth model system. The observation of loss in viability during heat shock at pH 5.4 in beef, but not in broth, underlines the differences between the two systems.

The most significant difference between the two model systems is, however, the much higher D-values found in beef. These observations emphasize that broth experiments cannot be used directly to generate D-values for use in the meat industry as this would lead to severe underestimations.

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